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Spore Wall Structure of Some Japanese Species in Polypodiaceae s. st.

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ウラボシ科シダ類の胞子壁について

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Hannig が 1911 年に，シダ植物には胞子の最外層にタペータム組織から由来した周皮を持つ分類群と持たない分類群があることを発表した。そして前者には，オシダ科，チャセンシダ科やその近縁群が含まれ，これらの分類群では，周皮がつくる模様が分類の重要形質となっている。後者には，主にウラボシ科の種が含まれてきた。その後50年間，この説に関してはあまり検討されないまま，安易に多くの分類学者に利用されてきている。しかし近年，電子顕微鏡を利用した研究や，化学処理を用いて，ウラボシ科のシダ類にも，明らかに周皮を持つものがあることが確かめられてきている。

今回，走査電子顕微鏡と GMA 樹脂包埋の切片を用いて，この科の各種がどのような胞子壁の構造を持つか，9属15種で調査した。

その結果，周皮は観察した15種の全てに存在することが確認された。その存在様式に大別して2形がある。第1群は周皮が顕著に発達し，分類上の形質となり得るような模様をつくる場合で，第2群は透明な薄膜状で外膜の模様通りに単に覆っている場合である。第1群にはミツデウラボシ属（3種）とヒトツバ属（2種）が含まれる。ミツデウラボシでは厚さ2μで平滑な外膜の表面を茶褐色の小顆粒状の周皮が1.5μ程の厚さに沈着して，さらに刺状突起や腺状突起が散在している。ヒトツバでは厚さ2μの平滑な外膜の表面に，直径5μ内外の球体が点在していて，この球体と外膜全体を透明な薄膜が被っている。第2群の代表的なものは，エゾデンダ属（3種）とノキシノブ属（1種）である。オガサワラウラボシ（ノキシノブ属）では外膜に隆起部が生じ，厚さ7μ程になり，大きな網目模様を形成する。この外膜の表面に薄膜状又は断片状の周皮が沈着しているが光顕では確認することが困難である。エゾデンダ属でも同じ程度に外膜が肥厚するが網目状の模様はつくらない。サジラン属，マメヅタ属でも周皮は同様であるが，外膜の厚さが3～4μで，隆起部の高さはほぼ均一である。

クリハラン属（1種），イワヒトデ属（1種）とヌカボシクリハラン属（2種）では，これら2群の中間形の胞子壁を持っている。外膜の肥厚は2～3μで顆粒状の隆起がわず

かに観察される程度である。周皮は薄膜状又は透明な刺状突起が点在する程度で顕著な模様はつくらない。以上のように周皮の発達と外膜の発達には密接な関係がみられる。周皮が発達し顕著な模様を形成するものでは外膜は平滑で肥厚がみられない。外膜が発達し，顕著な模様を形成するものでは，周皮の発達はほとんどみられない。Bower (1928) は，周皮のあるものは，胞子の発達する段階で栄養源として，吸収されることを述べているが，今回観察された結果は，Bower の報告と関係があるようにも思われる。

胞子の表面模様は，従来分類学者に安易に，その模様の特徴だけを利用されてきているが，胞子壁のどの部分が模様を形成するかを，検討して，分類形質として利用すべき段階にきていると思われる。

Spore Wall Structure of Some Japanese Species in Polypodiaceae s. st.

Since Hannig (1911), it has been recognized that most spores in Polypodiaceae s. st. are characterized by the absence of perispore. For instance, Nayar and Devi (1964) studied 127 species of this family and stated that the absence of the perispore characterized this family. In Alston's system (1956), the absence or presence of perispore was used in combination with spore type and other characters to delinate the families of the Polypodiaceae s. l., and the Polypodiaceae s. st. was distinguised to possess the spores without perispore. On the other hands, Lloyd (1969) reported that spores of *Polypodium chnoodes* possessed a large winged hyaline, nearly transparent perispore surrounding the exine. Furthermore, Lugardon (1974) found a thin perispore covering the exine of *Polypodium serratum* by the electron microscope.

The present study deals with the spore morphology of 15 species belonging to 9 genera of the Polypodiaceae s. st., with the special references to the feature of spore surface and to the structure of spore wall in the light and the scanning electron microscope.

Materials and methods

The species investigated are listed in tab. 1 with their locality, spore size and ploidy. Spores were collected from fresh fronds and stored in the decicator till beginning of this study. For the scanning electron microscopy, fresh spores were coated with carbon and gold in the vaccum of 10^{-5} torr. The vouchers are deposited in the herbarium of the Department of Botany, National Science Museum, Tokyo (TNS), and the slides are located in the Biological Deparment of Nippon Dental University, Niigata. For the light microscopy, spores were embedded in 1% agar and fixed in carnoy's fluid and dehydrated in the following series of glycol methacrylate (GMA) ; 50%, 70%, 85%, 95%, and 100%. These specimens were then embedded in GMA

Table 1. Locality and spore size (without perispore) of species investigated, and their ploidy estimated from the chromosome numbers reported for the specimens from Japan

species	locality	size of spore (P×E) in μm	ploidy
Crypsinus hastatus (Thunb.) Copel.	Ikeshiro, Shizuoka Pref. TNS 321716	40.1×56.7	2X
C. veitchii (Bak.) Copel.	Mt. Shiraiwa, Nagano Pref. TNS 321718	43.1×61.3	4X
C. yakushimensis (Makino) Tagawa	Urauchigawa, Okinawa Pref. TNS 321717	38.1×56.2	2X
Pyrrosia linearifolia (Hook.) Ching	Sanpoku-machi, Niigata Pref. TNS 321719	49.2×71.2	2X
P. lingua (Thunb.) Farwell	Yakushima, Kagoshima Pref. TNS 321720	39.3×55.7	2X
Microsorium buergerianum (Miq.) Ching	Iriomote, Okinawa Pref. TNS 321721	40.1×60.1	?
M. scolopendria (Burmann) Copel.	Iriomote, Okinawa Pref. TNS 321722	43.1×62.2	2X
Colysis elliptica (Thunb.) Ching	Hooraizisan, Aichi Pref. TNS 321723	30.3×43.2	2X
Loxogramme saziran Tagawa	Monobe, Koochi Pref. TNS 321724	43.5×67.6	4X
Neocheiropteris ensata (Thunb.) Ching	Mitsuishi, Chiba Pref.	36.5×53.7	4X
Lemmaphyllum microphyllum var. *obovata* C. Chr.	Katsuudake, Okinawa Pref. TNS 321725	49.3×69.3	2X
Lepisorus boninensis (Christ) Ching	Chichijima, Tokyo Pref. TNS 321726	31.8×48.7	2X
Polypodium fauriei Christ	Hinoemata, Fukushima Pref. TNS 321727	39.8×61.1	2X
P. formosanum Bak.	Yakushima, Kagoshima Pref. TNS 321728	26.7×47.4	2X
P. virginianum L.	Soounkyo, Hokkaido TNS 321729	45.7×67.3	2X

and polymerized at the temparature of 60°C for two or three hours, and sectioned at 2 microns with glass knife. All sections were stained with toluidin blue for two minutes and mounted with eukitt. For the measuring of spore size, spores were mounted with eukitt directly. Measurments were average of 100 spores in each species and were expressed as polar diameter × longest equatorial diameter in Tab. 1. The terminology used is taken mainly from Kremp (1965).

Observations

Crypsinus hastatus (Thunb.) Copel. and *C. yakushimensis* (Makino) Tagawa

Spores are brown. The exine is smooth, about 2 μm thick. Perispore blackish-brown, spines slender, 4–7 μm tall. In SEM, the perispore possess granules (ca. 2 μm in diam.) except spines. (plates, A–3, 4, D–6, E–1)

C. veitchii (Bak.) Copel.

Spores are brown, exine is smooth, about 2 μm thick. Perispore blackish brown granulate, ca 2 μm thick. The form and size of granules of perispore varies and papillose or spinose processes are densely scattered on the surface of exine. (plates, A–1, 2, D–3)

Pyrrosia lingua (Thunb.) Farwell

Spores are yellow. The section shows densely spaced verrucae (about 6 μm in diam.) on the homogeneous exine. A thin membrane confirmed also in SEM and stained with toluidin blue covers these processes and exine. (plates, A–5, 6, D–5)

P. linearifolia (Hook.) Ching

Spores are yellow. The exine is smooth, homogeneous and 2.5 μm thick. Perispore is verrucose, covered with membrane as in *P. lingua*. (plates, A–7, 8, D–2)

Perispores found in above mentioned 5 species, can be detached from the exine surface after treatment of KOH or NaClO.

Microsorium buergerianum (Miq.) Ching and *M. scolopendria* (Burmann) Copel.

Spores are yellow. The exine is granulate, and *M. scolopendria* has thicker (ca. 3 μm) and more complex granulate exine than *M. buergerianum*. Fragmentary perispore can be loosed from exine after the treatment of certain alkaline solutions. (plates, B–1, 2, 5, 6, D–1, 8)

Neocheiropteris ensata Christ

The exine is granulate and 2.5 μm thick, and deeply stained spinules are found on the exine.

Colysis elliptica (Thunb.) Ching

Spores are yellow. The exine is faintly granulate, and 2 μm thick. Fragmentary or spinulate perispore is observed sometimes on the exine as well as *Microsorium* and *Neocheiropteris*. (plates, B–3, 4)

Loxogramme saziran Tagawa

Spores are yellow. The exine is about 3 μm thick and homogeneous, and forms scrobiculate ornamentation. (plates, B–7, 8)

Lepisorus boninensis (Christ) Ching

Spores are yellow. The exine is 3–6 μm thick and forms rough reticulum-like ornamentation. The features of exine surface of this species seems to be related to those of spores in diploid species in this genus reported in Mitui,

1971. (plates, C-1, 2, D-9)

Polypodium virginianum L.

Spores are yellow. The exine (4-7 μm thick) is cristate. The perispore is not observed in section, but in SEM many lines of thin membrane are observed at the base of crista. (plates, C-3, 4, E-3)

P. fauriei Christ

Spores are yellow. The exine is about 3-4 μm thick, rugulate. Ridges are longer and more straight in the lateral side than in the distal face. (plates, C-5, 6)

P. formosanum Bak.

Spores are yellow. The exine is about 3 μm thick, areolate. In SEM, membranous perispore covers the surface of exine and this membrane is also found by light microscope. (plates, C-7, 8, D-7)

Discussion

Kawasaki (1970) classified bilateral spores of ferns into 6 groups according to their external characters. It was stated in his paper that the genera of Polypodiaceae s. st., were closely related to each other, and this family was uniformed and a comparatively lower one in evolutionary stage in the view point of spore morphology. However, his classification of spore types seems to be merely mechanical to me, because he takes no notice of what part of spore wall forms the ornamentation of spores. Nayar and Devi (1964) pointed out that the spores of Polypodiaceae s. st. were devoid of a perispore and were comparatively larger than those of the other major groups of ferns with monolete spores. Furthermore, they stated that the exine was markedly thick (3-6 μm) and verrucose, areolate and spinulate type in many cases in this family. The spines found in a spore of *Crypsinus* and thin outer membraneous layer of a spore in *Pyrrosia* were described as a part of exine. From the point of these exine structures, they concluded that the Pleopeltoid, the Polypodioid, the Microsorioid and the Pyrrosoid ferns appeared to be independently evolved rather than one derived from other, and the Crypsinoid ferns exhibited affinities to the Microsorioid genera.

On the other hands, the perispore as is shown in the present investigation, surrounds the exine in this family and forms distinctive ornamentations in *Crypsinus* and *Pyrrosia*. Spores of other genera have also thin membraneous perispore covering the exine as shown in *Polypodium serratum* by Lugardon (1974), notwithstanding the thick exine forms the distinguishable ornamen-

tations of spores. As far as the present study, there seems to be a correlation between the thickness of exine and the developmental degree of the perispore. For instance, in *Crypsinus*, spores have a smooth and thin exine and well developed brown, spinulate or granulate perispore. These perispores are loosed easily from exine by KOH and other alkaline solution similarly to the typical perispore of Dryopteroid and others. The exine of *Crypsinus* shows faint foveolate ornamentation in SEM, resembling to that of the early developmental stage of tetrads in *Lepisorus* (Mitui, 1971) (plate, E-2.). In *Pyrrosia*, spores have also thin and smooth exine and their perispores are composed of characteristic hyaline membrane and verrucae on the exine. In *Microsorium buergerianum*, *Colysis* and *Neocheiropteris*, thin exine (2–3 μ) is granulate, and fragmentary or sometimes spinulate perispores are observed on the exine. *Microsorium scoropendria* has a thicker exine and more complex ornamentation than *M. buergerianum*. *Polypodium* and *Lepisorus* have the thickest exine in the material investigated and their perispores are like a thin hyaline membrane covering merely the exine which forms sculpture of spores. The perispore is obscure in the light microscope but membraneous or fragmentary perispores are observed in SEM micrographs of some spores in *Lepisorus* (plates, E-5, 6). Hyaline perispores are also confirmed in light microscope in *Polypodium formosanum*, and the features resemble to the hyaline membrane of *Pyrrosia* as mentioned above. The winged perispore studied previously in *Polypodium chnoodes*, considered to be a well developed type related to the perispore of *P. formosanum* judging from the description of Lloyd (1969).

The spores investigated in the present study can be devided into four spore types according to the features of perispore and the sculptur of exine; (1). The Crypsinus type; Colored perispore forms ornamentation of spores, (2). The Pyrrosia type; Hyaline perispore and spherical bodies of perispore forms ornamentation, (3). The Microsorium type; Thin exine (2–3 μm) is granulate and perispores are fragmentary or spines. *Microsorium*, *Colysis* and *Neocheiropteris* have spores of this type. *M. scoropendria* shows several affinities to the following type. (4). Polypodium type; Thick exine (over 3 μm) forms ornamentation and perispore is a hyaline membrane in many cases. *Loxogramme*, *Lemmaphyllum*, *Lepisorus* and *Polypodium* have spores of this type.

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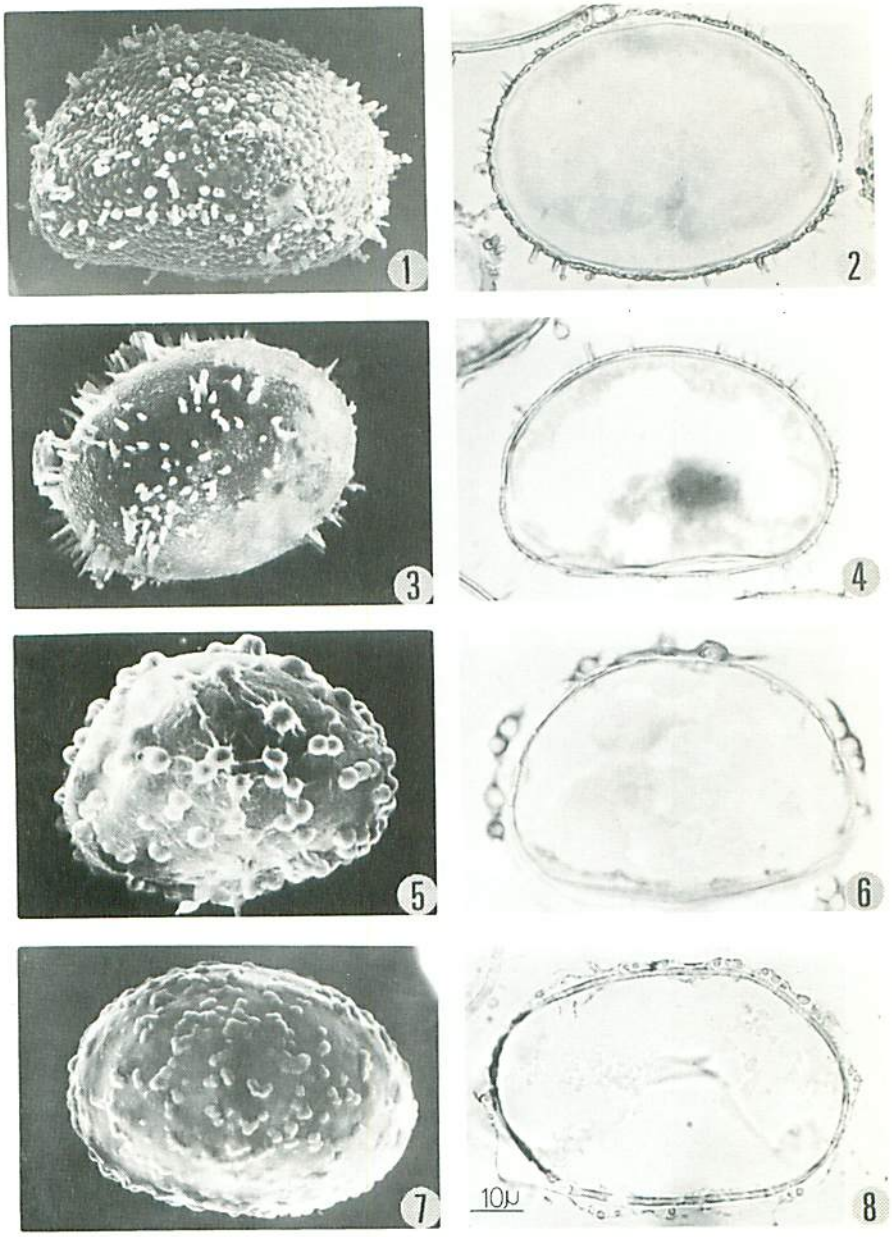

Plate A. Spores and sections of spore wall
1, 2. *Crypsinus veitchii* 3, 4. *C. yakushimensis* 5, 6. *Pyrrosia lingua*
7, 8. *P. linearifolia*

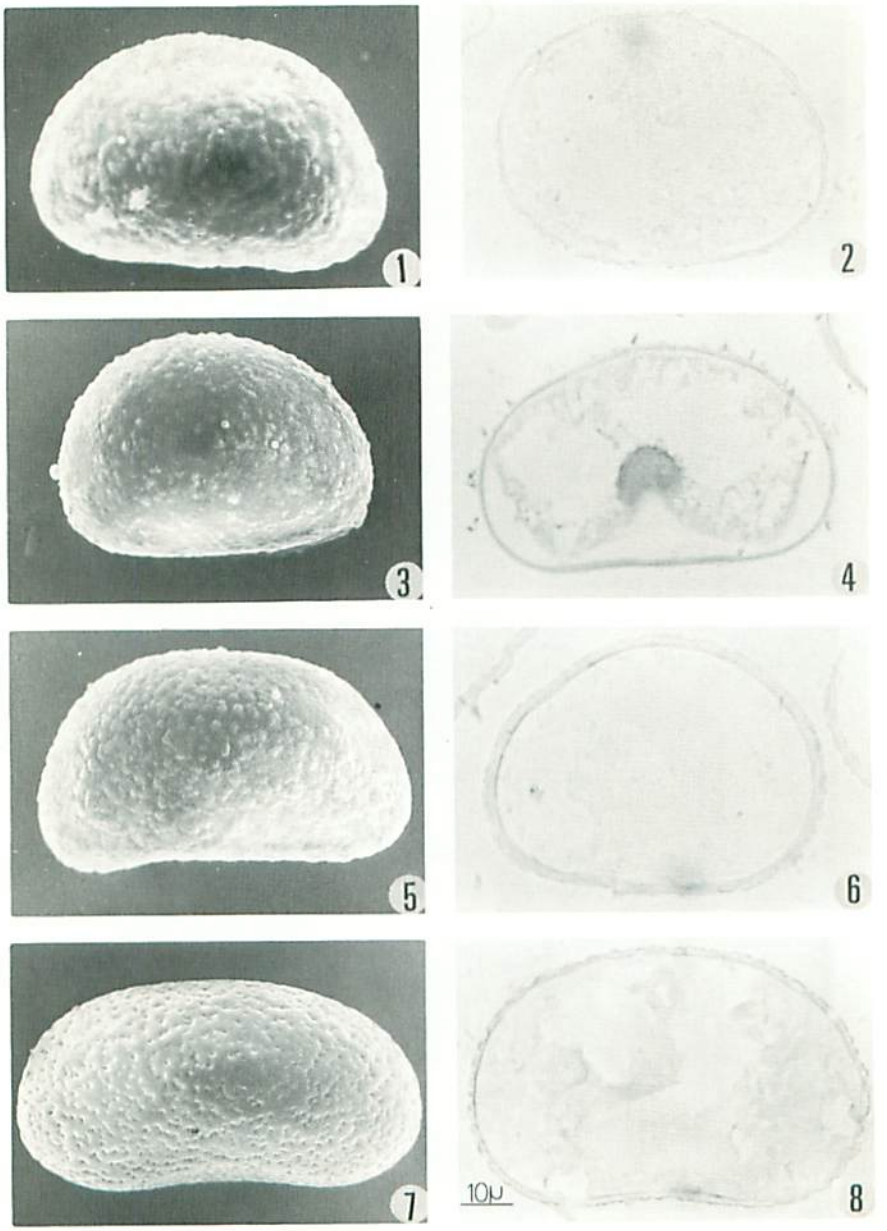

Plate B. Spores and sections of spore wall
1, 2. *Microsorium buergerianum* 3, 4. *Colysis elliptica* 5, 6. *Microsorium scolopendria* 7, 8. *Loxogramme saziran*

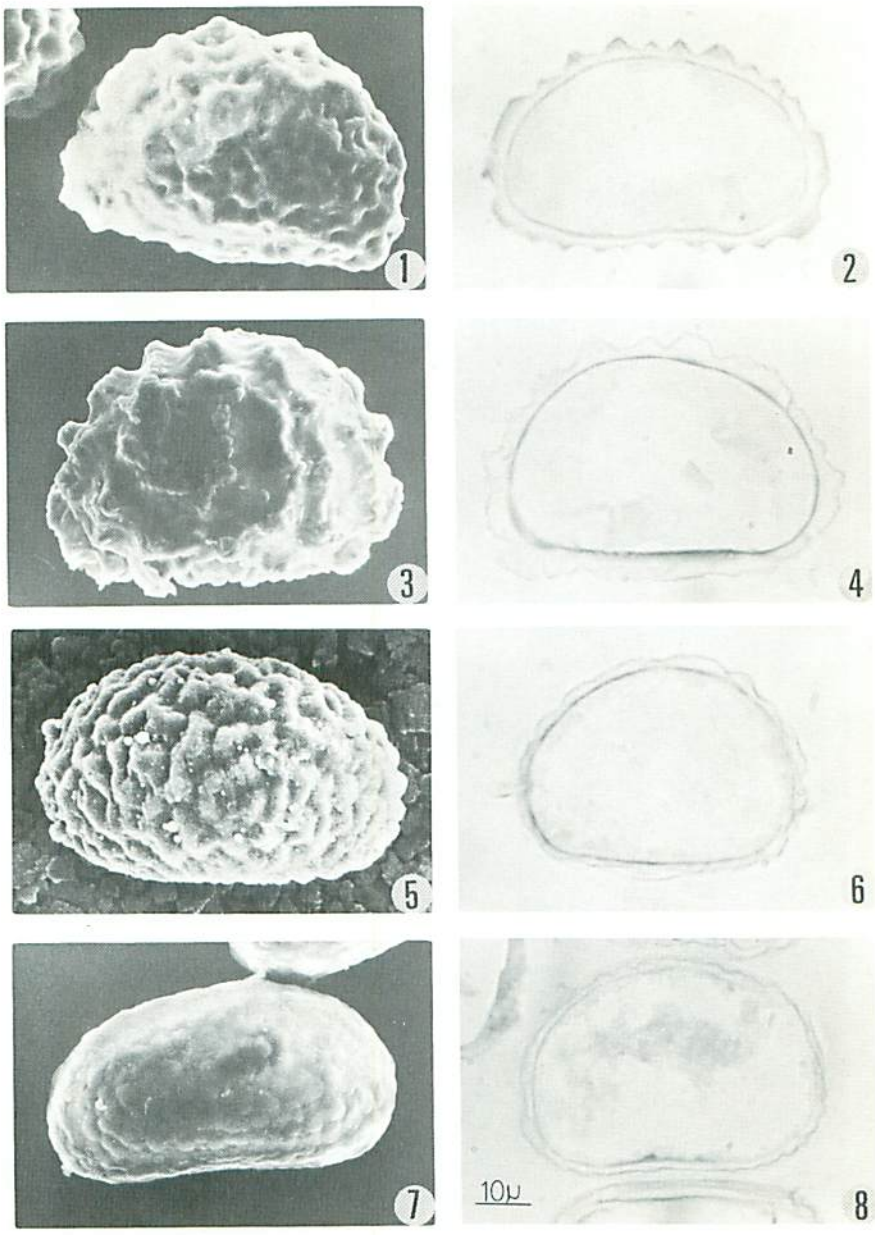

Plate C. Spores and sections of spore wall
1, 2. *Lepisorus boninensis* 3, 4. *Polypodium virginianum* 5, 6. *P. fauriei*
7, 8. *P. formosanum*

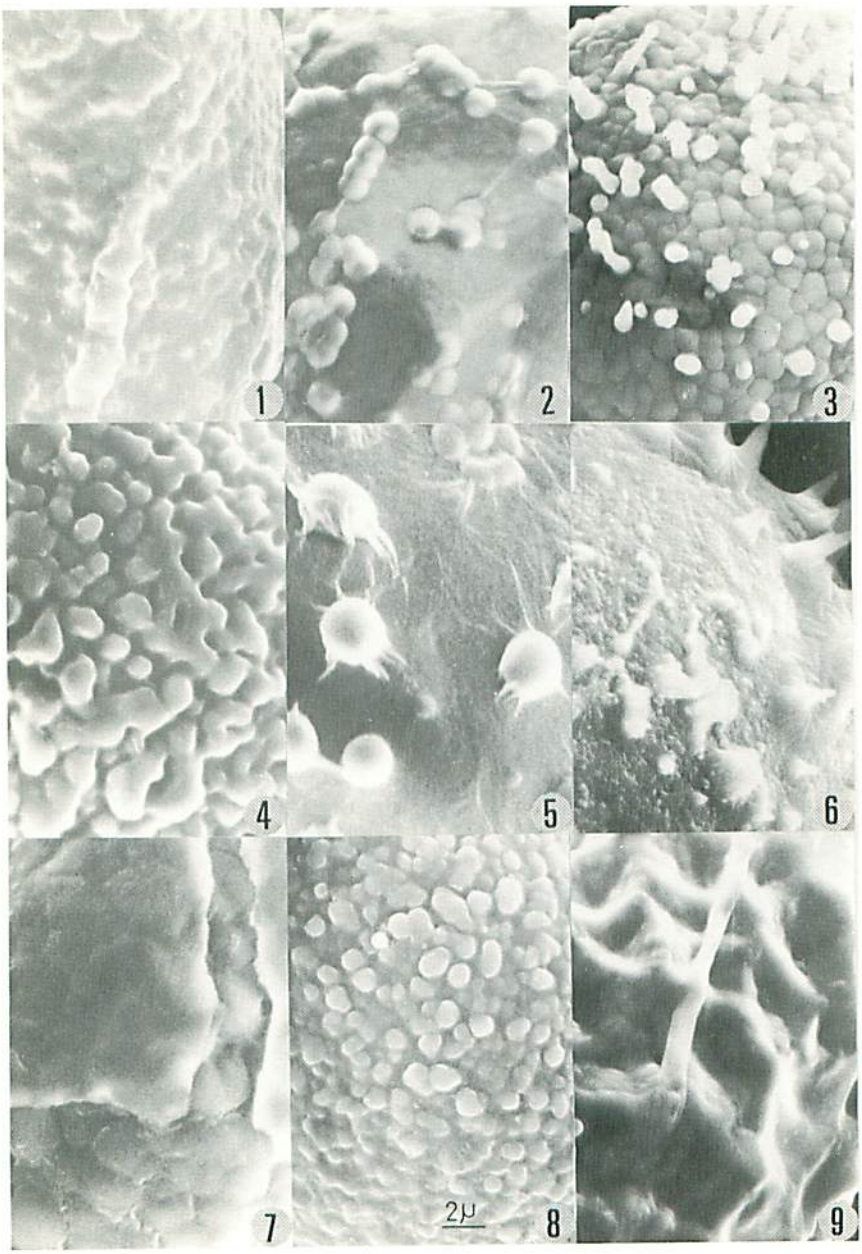

Plate D. SEM micrograph of spore surface
1. *Microsorium buergerianum*, exine
2. *Pyrrosia linearifolia*, perispore
3. *Crypsinus veitchii*, perispore
4. *Lemmaphyllum microphyllum* var. *obovata*, exine
5. *Pyrrosia lingua*, perispore
6. *Crypsinus hastatus*, perispore
7. *Polypodium formosanum*, exine
8. *Microsorium scolopendria*, exine
9. *Lepisorus boninensis*, exine

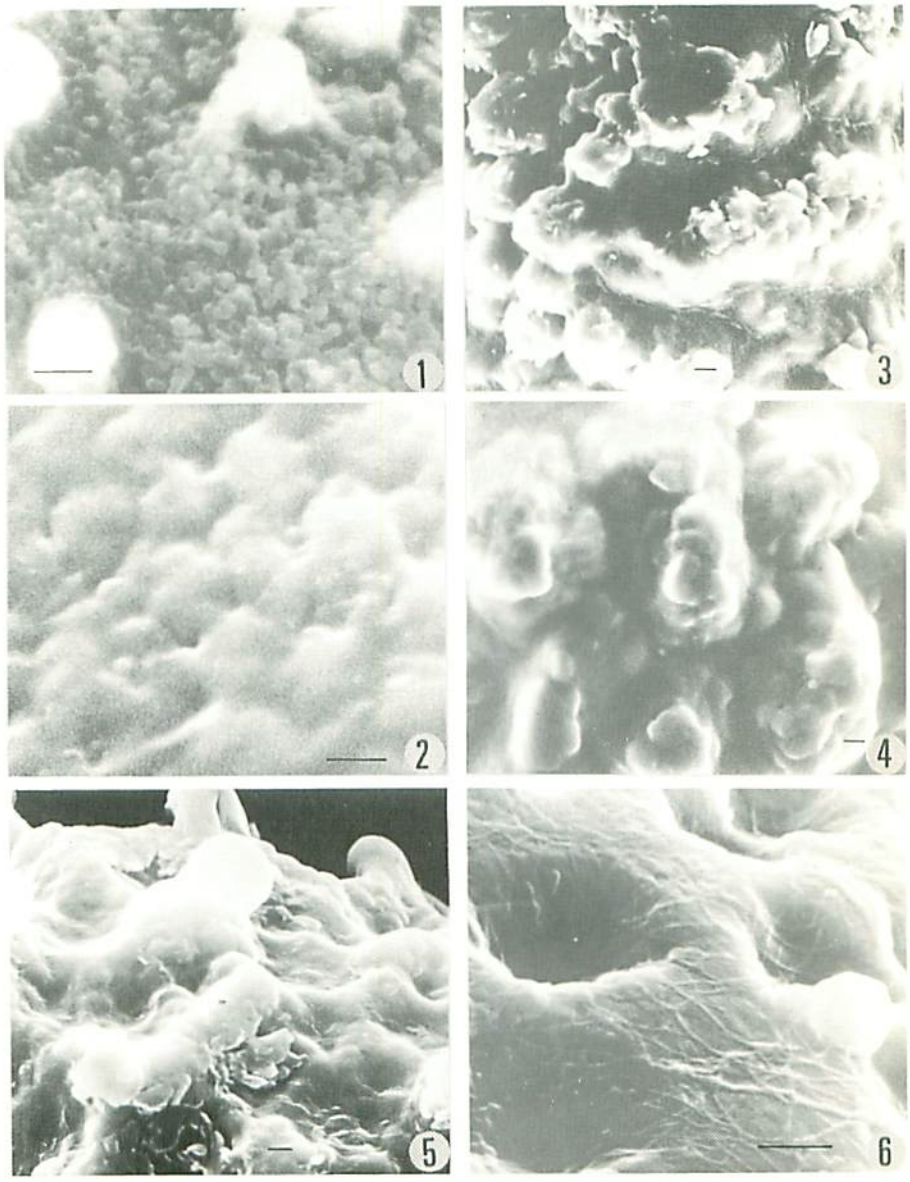

Plate E. All scales show 1 μm.
1. Perispore of *Crypsinus hastatus* 2. Exine surface of *C. hastatus* after removing perispore by NaClO 3. Surface of perispore in *Polypodium virginianum* 4. Surface of exine in *P. virginianum* after removing perispore with NaClO 5. Surface of spore in *Lepisorus hachijoense*, showing fragmentary perispore 6. Surface of spore in *L. clathratus*, showing lines of hyaline perispore